Effect of acupuncture at three different acupoints on electrical activity of gastric distention-affected neurons in rat medial vestibular nucleus

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Abstract

OBJECTIVE: To observe the effect of gastric distention (GD) and acupuncture at three different acupoints on the spontaneous discharge of neurons in the medial vestibular nucleus (MVN), and to clarify the specific function of the MVN in the central integration mechanism underlying acupuncture regulation.

METHODS: GD was conducted using a balloon inserted in the stomach cavity, and acupuncture was performed separately at each of three acupoints: Zusanli (ST 36), Quchi (LI 11), and Weishu (BL 21). The effect of acupuncture and GD on the spontaneous discharge of MVN neurons was assessed using a glass microelectrode filled with a sodium acetate electrolyte solution containing 1% pontamine sky blue; the discharge signals from the neurons were amplified by the microelectrode amplifier and recorded in the Spike2 system.

RESULTS: GD and acupuncture significantly affected the spontaneous discharge of MVN neurons. Furthermore, acupuncture at Zusanli (ST 36) and Weishu (BL 21) was significantly more effective at altering the discharge of GD-responsive MVN neurons compared with GD-nonresponsive neurons.

CONCLUSION: GD and acupuncture at three different acupoints affected the electrical activity of MVN neurons. The MVN is involved in the central integration mechanism underlying acupuncture regulation of gastric functions. The effects of acupuncture on gastric function may therefore be mediated via these particular MVN neurons.

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Keywords: Acupuncture; Vestibular nucleus; Motion sickness; Electrical activity; Spontaneous discharge; Gastric distention

INTRODUCTION

The vestibular system affects balance and propriocept-
tion, and plays a key role in the visceral activities associated with balancing ability. The vestibular receptors send signals to the vestibular nuclei (VN) and other relevant nuclei, and integration between the vestibular system and other systems enables these receptors to regulate body postures and muscular tension to maintain body balance.\(^1\) Furthermore, visceral activities such as circulation and digestion are all rapidly and adaptively adjusted.\(^2\) Previous studies have found that acupuncture stimulation of specific acupoints can significantly alleviate the symptoms caused by motion sickness (MS), and efficiently relieve nausea, vomiting, and other symptoms.\(^3\) However, the central mechanism and the specific pathways by which acupuncture signals are transferred to the brain are still unclear.

As the VN play important roles in the transfer and processing of vestibular information, we investigated the effect of gastric distention (GD) and acupuncture at three different acupoints on the spontaneous discharge of neurons in the medial vestibular nucleus (MVN). The MVN is the main structure of the VN. We hypothesized that the MVN is involved in the central integration mechanism underlying the regulatory effect of acupuncture on gastric function. This study may provide a new direction for research on the gastric regulatory mechanisms of acupuncture.

MATERIALS AND METHODS

Experimental animals

Ninety healthy Sprague-Dawley rats of Specific pathogen free grade (all 4-month-old males weighing 250-300 g) were purchased from the Model Animal Research Center of Nanjing University (certificate of quality No. SCXK 2013-0005). Food and water were available ad libitum, and the rats were housed under controlled environmental conditions. All experimental manipulations were undertaken in accordance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals, published by the National Science Council, China.

Materials and drugs

Urethane was purchased from Shanghai Qingxi Chemical Technology Co., Ltd., (Shanghai, China). Pontamine sky blue was purchased from Sigma (Chicago, IL, USA). Anhydrous sodium acetate was purchased from Nanjing Chemical Reagent Co., Ltd., (Nanjing, China). Stereotoxic apparatus was purchased from David Kopf Instruments (Tujunga, CA, USA). The microelectrode manipulator was purchased from Narishige Co., (Tokyo, Japan). The extracellular electrophysiological record amplifier was purchased from A-M Systems (Chicago, IL, USA). The Micro 1401-3 biosignal collection and Spike2 analysis system were purchased from CED (London, UK). The H-KWDY-III temperature controller was purchased from Nanjing Quanshui Teaching Equipment Factory (Nanjing, China). The dental drill was purchased from Shanghai Alcott Biological Science Technology Company (Shanghai, China). The WD-1 glass microelectrode controller was purchased from Chengdu Instrument Factory (Chengdu, China). The glass microelectrodes (length 100 mm, outside diameter 1.2 mm) were purchased from Nanjing Quanshui Teaching Equipment Factory (Nanjing, China). Acupuncture needles (0.30 mm × 25 mm) were purchased from Suzhou Medical Supplies Factory (Suzhou, China).

Recording of neuron discharge

A glass microelectrode (tip 0.5-2 μm) was filled with a 0.5 mol/L sodium acetate electrolyte solution containing 1% pontamine sky blue (impedance 10-20 mΩ). The microelectrode controller was used to localize the microelectrode to the target nuclei, and the discharge signals from MVN neurons were recorded. After pre-amplification, discharge signals were routed into the biosignal acquisition system. The discharge signals from the neurons were amplified by the microelectrode amplifier and recorded in real time by the Spike2 system. When the neurons emitted spontaneous discharge signals, we adjusted the propeller up and down until the signal-noise-ratio was stable (the discharge amplitude stabilized at the same level, and the signal-noise-ratio was > 1/3), and then stopped the propulsion. The electrode tip was stopped at the discharging neuron, and data were recorded after the discharge stabilized. The signals were recorded at 1 min before intervention as the baseline. After intervention, when the spontaneous discharge stabilized and returned to baseline, the next intervention was started.

Gastric distention

After 12 h of fasting, the rats were anesthetized by 20% urethane (7 mL/kg). A trachea cannula was used to maintain smooth breathing. The upper abdomen was shaved before the abdominal cavity was cut open, and the liver was moved to expose the pylorus of the stomach and the superior segment of the duodenum. A 2 mm incision was made in the stomach 5-10 mm below the pylorus, and a small balloon made of flexible condom rubber was inserted through this incision into the stomach; the balloon was connected to a sphygmomanometer and a 10 mL syringe. After surgery, the rats were placed in prostrate position. When stable neuron discharges were recorded, the balloon was inflated with air using the syringe until the blood pressure reading reached 30 mm Hg. After 30 s of continuous distention, the balloons were quickly deflated until the blood pressure reading returned to 0 mm Hg. Acupuncture intervention was performed after the neuron discharge background returned to baseline.

Medial vestibular nucleus localization

Rats were fixed onto the stereotaxic apparatus in pros-
tate position, and underwent routine craniotomy as follows. In brief, the anterior and posterior fontanelle were exposed and adjusted to the same level. According to the Paxinos and Watson rat brain stereotaxic atlas, the coordinates of the MVN were: $-10.56$ to $-12.3$ mm (AP), $0.5$ to $2.0$ mm (L), $7.0$ to $8.2$ mm (H). A dental drill was used to perform craniotomy at the appropriate position, and surgical microscissors and microforceps were used to peel off the dura to expose the brain tissue. The surgical positions were then covered by warm liquid paraffin to protect against drying. During the experiments, an electric hot plate was used to maintain the animal’s body temperature at $(37.0 \pm 0.5)$ °C. After surgery, the animals were allowed to rest for 30 min until all indices stabilized.

**Group and stimulation**

Zusanli (ST 36), Quchi (LI 11), and Weishu (BL 21) were used as acupoints on the left side. To eliminate the effect of the order in which the acupoints were investigated, we stimulated the acupoints in random order. The needle twirling rate was $120-180$ twirls/min, and each acupoint was stimulated for 1 min. The next acupoint stimulation was not started until the neuron discharge recovered to baseline.

**Histological localization**

After each experiment, a digital display direct current stabilized voltage supply stimulator was used to supply converse direct current ($10 \mu A$, $20$ min) to the microelectrode, and the pontamine sky blue in the glass microelectrode was sent via microelectrophoresis to the electrode tip recording point. The brains were then removed and fixed in $4\%$ paraformaldehyde. After 1 week, $40$ to $60$ μm-thick brain sections were made using a frozen section instrument, and the location of the microelectrode tip was checked.

**Data collection**

The neuron discharge signals were preamplified and sent into a multichannel biosignal collection system for data collection and analysis by the Spike2 system. Using discharge frequency (DF, spikes/s) as the observation index, the DF before intervention was used as the baseline (DF$_0$) and the period of intervention was designated as DF. The DF changing rate (R$_{DF}$) was calculated as $(DF_0 - DF) / DF_0 \times 100\%$. The absolute value of R$_{DF}$ ≥ $15\%$ was regarded as excitation/inhibition, and R$_{DF}$ < $15\%$ was regarded as "no change".

**Statistical methods**

Data were analyzed with SPSS software version 18.0 (Chicago, Illinois, USA). Data were expressed as mean ± standard deviation. Data before and after acupuncture were compared via paired t-testing. Counting data were compared via the χ² test. Differences with $P < 0.05$ were considered to be significant.

**RESULTS**

**Effect of gastric distention on the spontaneous discharge of medial vestibular nucleus neurons**

The electrical discharges from a total of 141 MVN neurons were recorded from 70 rats. In the unstimulated state, the average DF was $(19 \pm 10)$ spikes/s. After GD, a total of 61 responsive neurons were detected ($57$ excited, $4$ inhibited), and the DF during GD stimulation significantly increased to $(24 \pm 17)$ spikes/s ($P < 0.01$, Figure 1A). The effect of GD on the spontaneous discharge of MVN neurons was dominated by excitation (Figure 1B).

**Effect of acupuncture on the spontaneous discharge of medial vestibular nucleus neurons**

In the MVN neurons investigated, the DFs during acupuncture at Zusanli (ST 36), Quchi (LI 11), and Weishu (BL 21) were significantly increased from baseline ($P < 0.01$, Table 1). Acupuncture signals from Zusanli (ST 36) (Figure 2A), Quchi (LI 11) (Figure 2B), and

![Figure 1](https://via.placeholder.com/150)

Figure 1 Electrophysiological responses of medial vestibular nucleus (MVN) neurons to gastric distention (GD)

A: effects of GD on spontaneous discharge of MVN neurons, $P < 0.01$, compared with baseline (pre-GD). B: GD stimulation had an excitatory effect on the spontaneous discharge of MVN neurons.
Weishu (BL 21) (Figure 2C) stimulated the MVN neurons.

Table 1 Effect of stimulation at three different acupoints on the spontaneous discharge of medial vestibular nucleus neurons

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Time</th>
<th>Discharge frequency (spikes/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zusanli (ST 36)</td>
<td>49</td>
<td>Baseline</td>
<td>20±15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>During intervention</td>
<td>22±17*</td>
</tr>
<tr>
<td>Quchi (LI 11)</td>
<td>44</td>
<td>Baseline</td>
<td>20±15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>During intervention</td>
<td>22±17*</td>
</tr>
<tr>
<td>Weishu (BL 21)</td>
<td>48</td>
<td>Baseline</td>
<td>19±12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>During intervention</td>
<td>23±14*</td>
</tr>
</tbody>
</table>

Note: the discharge frequency during acupuncture at Zusanli (ST 36), Quchi (LI 11), and Weishu (BL 21) were significantly increased from baseline. *P < 0.01 compared with respective baseline values.

**Comparison of acupuncture effecting on gastric distention-stimulated medial vestibular nucleus neurons**

We compared the effects of acupuncture on diverse MVN neurons, and found that Zusanli (ST 36) and Weishu (BL 21) were significantly more effective at increasing activity in GD-responsive MVN neurons than in GD-nonresponsive MVN neurons (P < 0.05, P < 0.01, Table 2).

**Medial vestibular nucleus histological localization**

We compared our recording site with the known location of the MVN (red area) on brain coronal sections (Figure 3A). We found that our staining technique identified the recording site, and we assessed whether those sites deviated from the MVN (Figure 3B). If deviation was found, the relevant data from those animals were excluded.

**DISCUSSION**

Acupuncture has a significant effect on gastrointestinal motility, and can effectively cure diseases or symptoms caused by abnormal gastrointestinal motility, such as nausea, vomiting, and functional dyspepsia.14-17 Although the neural pathways and central mechanisms underlying the intracephalic transmission of acupuncture signals are not well understood, the vestibular system may be involved in the central regulation. In the present study, we found that stimulating the stomach with GD altered the spontaneous discharge of MVN neurons, which was generally manifested as increased excitation, indicating that MVN neurons are involved in gastric function regulation. We investigated MVN neuronal activity after stimulating the acupoints Zusanli (ST 36) (lower limb), Quchi (LI 11) (upper limb), and Weishu (BL 21) (the back). The results showed that acupuncture-induced signals affected MVN neurons, suggesting that the MVN is involved in the transmission of the acupuncture effect. Existing knowledge about the role of the vestibular system is limited to regulation of eye movement, head movement, and balance by the brainstem and spinal cord neurons.14-16 Recent studies indicate that the vestibular system not only plays an important regulating role in body balance and proprioception, but is also involved in the rapid regulation of visceral activities, such as the digestive, respiratory, and circulatory systems.17-20 The VN are the initial zone where the central nervous system processes the information transmitted to the vestibular system. After the vestibular organs are stimulated, VN neurons are excited, and the VNs emit and project to the visceral-associated nuclei, inducing changes in sympathetic and parasympathetic activities.21,22 Abnormal VN activity can impact the balance and stability of the internal environment; MS is a common condition caused by vestibular system abnormality.23 The main clinical manifestations of MS are vegetative reactions, including dizziness, nausea, vomiting, and gastrointestinal discomfort.24 It is believed that MS induced by motor vehicles (e.g., vehicles, boats, and airplanes) is associated with abnormal vestibular system activity. Previous reports on clinical treatment showed that stimulation of specific acupoints could effectively alleviate MS symptoms, such as nausea and vomiting;25-27 this could serve as an experimental basis for acupuncture-based treatment of conditions caused by abnormalities of the vestibular system.

Our study indicated that MVN neurons were divided into GD-responsive and GD-nonresponsive neurons. We found that the acupoints Zusanli (ST 36) and Weishu (BL 21) had a significantly greater effect on GD-responsive MVN neurons than on GD-nonresponsive neurons. Zusanli (ST 36) and Weishu (BL 21) are the two acupoints most commonly used for clinical treatment of gastrointestinal diseases, and show outstanding regulatory effects on symptoms associated with gastrointestinal discomfort.27-29 The difference in their actions may be attributed to the activation of specific MVN neurons. Earlier studies show that the VN has direct or indirect fiber projection with many intracephalic nuclei, and has a feedback loop with the nuclei in areas that regulate the viscera.26-27 The cortex at the lower cerebral margins is also involved in the MVN-regulated gastric motion.28 Therefore, after stimulation, the VN have incoming and outgoing links with several brain areas and nuclei that may play important roles in visceral reflection, transfer of proprioceptive information, and posture control. After acupuncture signals are transmitted to MVN neurons, they may be transmitted to nuclei and brain areas associated with gastric motility, and thereby contribute to the regulation of relevant processes. We hypothesize that Zusanli (ST 36) has a greater regulatory effect on gastrointestinal systems compared with Weishu (BL 21) or Quchi (LI 11), which may be due to its ability to stimulate specific MVN neurons.
Table 2 Comparison of the effect of stimulation at three different acupoints on gastric distention (GD)-stimulated medial vestibular nucleus neurons

<table>
<thead>
<tr>
<th>Group</th>
<th>GD-stimulated neurons</th>
<th>Effective (n)</th>
<th>Ineffective (n)</th>
<th>Efficient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zusanli (ST 36)</td>
<td>GD-responsive</td>
<td>14</td>
<td>8</td>
<td>70.00 a</td>
</tr>
<tr>
<td></td>
<td>GD-nonresponsive</td>
<td>8</td>
<td>19</td>
<td>29.63</td>
</tr>
<tr>
<td>Quchi (LI 11)</td>
<td>GD-responsive</td>
<td>9</td>
<td>11</td>
<td>45.00</td>
</tr>
<tr>
<td></td>
<td>GD-nonresponsive</td>
<td>6</td>
<td>18</td>
<td>25.00</td>
</tr>
<tr>
<td>Weishu (BL 21)</td>
<td>GD-responsive</td>
<td>15</td>
<td>4</td>
<td>78.95 b</td>
</tr>
<tr>
<td></td>
<td>GD-nonresponsive</td>
<td>7</td>
<td>22</td>
<td>24.14</td>
</tr>
</tbody>
</table>

Notes: Zusanli (ST 36) and Weishu (BL 21) were significantly more effective at increasing activity in GD-responsive neurons than in GD-nonresponsive neurons. ^P < 0.05, ^P < 0.01, compared with GD-nonresponsive neurons.

Figure 2 Effect of acupuncture at three different acupoints on the spontaneous discharge of medial vestibular nucleus neurons. A: acupuncture at Zusanli (ST 36); B: acupuncture at Quchi (LI 11); C: acupuncture at Weishu (BL 21).
The responses of MVN to external stimuli were relatively singular, and the MVN responded to GD and acupuncture mainly in the form of excitation or no response, but there was no significant dose-inhibition effect detected. The MVN may connect to the nuclei associated with gastric function regulation and transfer information, thus indirectly playing a role in visceral reflexion. In our view, the MVN is likely to be involved in the transfer of stimulating signals in the central mechanism underlying acupuncture regulation of gastric function.

In the present study, we showed that GD and acupuncture affect MVN neuronal activity, suggesting that the MVN is involved in the central pathway of acupuncture-mediated gastrointestinal regulation. The relationship between the therapeutic efficacy of acupuncture and activity in the central nervous system and the peripheral field has not been widely investigated, and further study is needed to identify the specific neural pathways involved.

REFERENCES

Yates BJ, Catanzaro MF, Miller DJ, McCall AA. Integration of vestibular and emetic gastrointestinal signals that produce nausea and vomiting: potential contributions to motion sickness. Exp Brain Res 2014; 232(8): 2455-2469.


